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TEST CARTRIDGE FOR
EVALUATING BLOOD PLATELET FUNCTIONALITY
CROSS-REFERENCE TO OTHER APPLICATION

BACKGROUND OF THE INVENTION

1. Field of the Invention

5 The present invention relates to an apparatus for evaluating blood platelet functionality. More specifically, the invention relates to an improved multicell cartridge for use in evaluating blood platelet functionality and method for using the same.

10 2. Description of the Prior Art

It has been observed that blood platelets play a significant role in the clotting or coagulation of whole blood. When platelets are activated, they shorten the clotting time of the blood. This shortening is related to the initial status of the platelets and platelet disfunction is considered a leading cause of post-surgical bleeding following cardiopulmonary bypass surgery.

Blood platelet functionality is conventionally determined by mixing blood and a clot promoting reagent such as kaolin in a buffer solution. This is done in a series of test cells incorporated in a test cartridge. After adding the clotting reagent, the blood/kaolin solution in each cell is agitated to activate the platelets to promote clotting. The degree of agitation of the blood sample in each cell differs one from the other. As described in U.S. Pat. No. 5,314,826, the clotting time is proportional to the degree of agitation. By comparing clotting times of aliquots of the blood as a function of degree of agitation, the blood platelet functionality can be determined. This process and the apparatus for carrying it out are disclosed in detail in

U.S. Pats. Nos. 4,599,219 and 5,314,826. Where necessary for a further understanding of the present invention, the disclosures in these two patents are incorporated by reference herein.

5 Chemical platelet activators or reagents are well-known in the art. One such activator, 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine, a biologically active phospholipid, is disclosed in Demopoulos, et al., J. Biol. Chem., 1979; 254:9355-8. This platelet
10 activator or reagent, often referred to as a platelet activating factor, enhances the ability of active platelets to effectively participate in the blood clotting reaction and thereby shorten the clotting time of the blood. If the platelets are inactive or not
15 functioning normally, the activator will have a lessened or no effect on the clotting time.

OBJECTS OF THE INVENTION

It is the principal object of the present invention to provide an improved platelet functionality test
20 cartridge that facilitates the evaluation of functional platelets in a blood sample.

Another object of the present invention is to provide a test cartridge that, upon receipt of blood sample aliquots therein, provides clotting results that
25 are predictive of platelet activity.

SUMMARY OF THE INVENTION

Sub A ~~In accordance with the foregoing objects, the present invention is embodied in a cartridge having a plurality of test cells. Each cell is adapted for
30 receiving an aliquot part of a blood sample. A measured amount of clotting reagent is applied in the reaction chamber of each cell as a dried fill. The amount of reagent in each cell differs from the amount of reagent~~

in each other cell, at least one of the cells containing
 no platelet activating reagent. Additionally, amounts of
 heparin or protamine may be added in each cell either as
 a liquid or dried fill. The cells also include a
 5 clotting reagent such as kaolin which on use of the
 cartridge is inserted into the reaction chamber and mixed
 with the blood and platelet activation reagent. The
 relative clotting times of the samples in each of the
 cells is measured and, when compared to a standard and
 10 each other, determines the platelet functionality of the
 blood sample.

The cartridge and method of determining platelet
 functionality is useful in connection with open heart and
 cardiopulmonary surgery wherein the blood condition of
 15 the patient must be closely monitored.

DESCRIPTION OF THE DRAWINGS

Fig. 1 is a front elevation view of a multicell
 cartridge embodying the present invention.

Fig. 2 is a section view taken substantially in the
 20 plane of line 2-2 on Fig. 1.

Fig. 3 is a diagram showing the relationship of
 platelet activating factor concentration to clotting
 time.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Sub A2
 25 ~~The present invention is embodied in a test~~
 cartridge 10 having a plurality of test cells 11,
 preferably six such cells, depending from and integral
 with a cartridge plate 12 having a front depending skirt
 or panel 14. The cartridge is adapted to be inserted
 30 into a test apparatus such as shown and described in
 detail in U.S. Pat. 4,599,219 for the determination of
 clotting time of an aliquot blood sample inserted into
 each test cell 11 as described in detail in said patent.

Each cell is formed by a downwardly tapered tube 15 defining an inwardly projecting annular seat 16 intermediate its ends and in turn defining an upper sealing surface 18 and a lower sealing surface 19. A resilient flexible sliding plug 20 is positioned in the lower end of the tube 15 while a plunger 21 defined by a plunger shaft 22 and a sealing washer or disk 24 is positioned in the upper portion of the tube. The sealing washer 24 seats against the upper sealing surface 18 of the annular seat and defines with the plug 20 a lower clotting reagent chamber 25. The tube 15 defines above the washer 24 a blood receiving reaction chamber 26. At its upper end the plunger 21 defines a flag 28 and is adapted for engagement by the test machine (not shown).

A clotting reagent 29, such as kaolin in a buffered, bacteriostatic solution, is contained in the clotting reagent chamber 25 above the plug 20 and below the seal washer 24. When the cartridge is used, the plunger 21 of each cell is lifted and the plug 20 is pushed upwardly, thereby forcing the clotting reagent into the blood sample contained in the upper cell reaction chamber 26 to initiate clotting.

Sub A3 ~~In accordance with the present invention, a measured amount of a chemical platelet activating factor or reagent 30 is provided in the top or upper reaction chamber 26 as a dried fill. This platelet activating factor composition is dissolved in the blood sample when the blood sample is introduced into the clotting chamber 26 and the clotting reagent 29 added and mixed therein. Additionally, selected amounts of heparin or protamine may be utilized as a dried fill in the reaction chamber 26, depending on the chemical procedure to be utilized.~~

In order to provide a series of differing clotting times, the amount of platelet activating factor in each

cell differs from the amount in each other cell. In the first two cells 11A and 11B (as shown in Fig. 1), no platelet activating factor is utilized. In each succeeding cell 11C, 11D, 11E and 11F, increasing amounts
 5 of platelet activating factor or reagent are utilized.

The preferred platelet activating factor is the compound 1-O-alkyl-2-acetyl-sn-glycerol-3-phosphorylcholine, a biologically active phospholipid. Other factors or compounds which may be used are
 10 collagen, epinephrine, ristocetin and arachidonic acid. Fills of the preferred platelet activating factor, 1-O-alkyl-2-acetyl-sn-glycerol-3-phosphorylcholine, are prepared by mixing the factor with a saline (NaCl) solution containing 0.25% bovine serum albumin, and
 15 diluting with deionized water to the desired factor concentrations. An amount of each solution of the desired factor concentration is placed in a cell and allowed to evaporate, leaving a solid or dry fill residue of the desired amount of platelet activating factor.
 20 Desired amounts of heparin and protamine may also be added and dried as a fill.

The clotting reagent, such as kaolin, is prepared as a 4% w/v suspension in hydroxyethylpiperazine ethanesulfonic acid buffer with 0.5m calcium chloride,
 25 and sodium azide as a bacteriostatic agent. The amount of 0.088ml of this clotting reagent is loaded into the reagent chamber 25 of each cell 11 of the cartridge 10.

In use, aliquots of 0.35ml per cell of a blood sample are dispensed into each cell. This results in
 30 platelet activating factor (PAF) blood concentrations illustratively shown in the following Table.

TABLE I

Cartridge PAF Concentrations					
Amount of PAF in Platelet Function--PAF Cartridge					
Cell A	Cell B	Cell C	Cell D	Cell E	Cell F
0.0 ng	0.0 ng	23 ng	116 ng	230 ng	2.76 μ g
Final Concentration of PAF in Blood					
0.0 nM	0.0 nM	1.25 nM	6.25 nM	12.5 nM	150 nM

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Sub A ~~After introducing the blood samples in each cell~~
 reaction chamber, the clotting reagent is inserted into
 each reaction chamber and the clotting time of the blood
 in each cell is determined. From the clotting time for
 each cell, the clot ratio is calculated. Clot ratio is
 the ratio of the clotting times for cells C, D, E and F
 compared to the average control clotting times, Cells 11A
 and 11B. Platelet function is expressed as a percentage
 of the maximum clot ratio response observed in a normal
 population. This value of a normal population response
 is known and can be used to compute the clot ratio
 percentage which is in turn indicative of the platelet
 functionality. Any appropriate desired calculation may
 be made from the relative clotting times in each cell.
 The platelet functionality can in turn be utilized to
 determine blood loss during surgery and the need for a
 blood transfusion. The platelet functionality further
 assists in managing heparin therapy during cardiac
 surgery.

EXAMPLE I
PREPARATION OF PLATELET
ACTIVATING FACTOR SOLUTIONS AND CELLS

1. Weigh out 62.5mg Bovine Serum Albumin (BSA) (Sigma Product #A-3803).
2. Weigh out 219mg NaCl.
3. Make up to 25ml with deionized water. This gives 0.25% BSA/0.15M NaCl. Leave until BSA is completely in solution.
4. Using a Hamilton syringe, pipette 50 μ l platelet activating factor 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine into a clean stoppered vial and allow to evaporate in a fume hood. Add 2ml BSA/NaCl solution and leave at least 1 hour. This working stock material is at 100 μ M.
5. Dilute the working stock platelet activating factor (PAF) in tenths serially down to 0.1 μ M with deionized water. 5 μ l of each of these solutions gives 1.25 μ M, 12.5 μ M, 125 μ M and 1250 μ M in 0.4ml blood, respectively.
6. The following amounts are added to the cells and result in the indicated blood concentration:

<u>Cell</u>	<u>Reagent Added</u>	<u>Concentration of PAF</u>
A	5 μ l BSA/NaCl	0 nM
B	5 μ l 0.1 μ M PAF	1.25 nM
C	5 μ l 1 μ M PAF	12.5 nM
D	5 μ l 10 μ M PAF	125 nM
E	2 μ l 100 μ M PAF	500 nM
F	5 μ l 100 μ M PAF	1250 nM

7. The water is allowed to evaporate, leaving a dry fill in each cell.
8. Using a sample of normal blood from a voluntary donor, and a cartridge prepared according to

EXAMPLE I, 0.4ml aliquots of blood were added to each cell and the clotting time of the blood in each cell was determined and plotted as Fig. 3.

5 As referred to above, the titration curve can be normalized by converting the clotting times to ratios. The clotting time of Cell A, with no platelet activating factor present, is the cell clotting time to which all other cell clotting times are compared. The ratio is
10 calculated by dividing the Cell A clotting time in seconds by each other cell clotting time in seconds. A clot ratio is then calculated as 1 minus the ratio of Cell A clotting time to other cell clotting times ($1 - \text{cellAtime}/\text{cellxtime}$). Data can also be presented in
15 terms of platelet function as a percentage of normal. This is calculated from the clot ratio by multiplying the clot ratio by 100 and then by a factor of 1.97 which has been determined by measuring the maximum platelet
20 activating factor response in 22 normal donors. These donors had no known platelet dysfunction and were taking no known medications.

 The test cartridge and method described herein are useful for providing a simple and rapid response point-of-care platelet function assay. This assay identifies
25 patients with excessive post-cardiopulmonary bypass blood loss who could benefit from further blood treatment and management.

 While a certain illustrative embodiment of the present invention has been shown in the drawings and
30 described above in detail, it should be understood that there is no intention to limit the invention to the specific form disclosed. On the contrary, the intention is to cover all modifications, alternative constructions and compositions, equivalents and uses falling within the

[illegible]